STUDIES IN NON-SPECIFIC COMPLEMENT FIXATION *

III. THE INFLUENCE OF SPLENECTOMY AND ANESTHETICS ON THE NON-SPECIFIC COMPLEMENT FIXATION SOMETIMES SHOWN BY NORMAL RABBIT AND DOG SERA

JOHN A. KOLMER AND RICHARD M. PEARCE

From the McManes Laboratory of Experimental Pathology and The John Herr Musser Department of Research Medicine, University of Pennsylvania, Philadelphia

This investigation was undertaken in the hope of obtaining some understanding of the part played by the spleen in hemolysis and of the mechanism by which the red cells develop an increased resistance to hemolytic agents after splenectomy. Antischkow[1] has shown that the feeding of rabbits with cholesterin leads to an increased deposition of anisotropic fats in the spleen. Eppinger[2] and King[3] have found that dog's blood after splenectomy shows an increase in total fats and cholesterin and a decrease in unsaturated fatty acids.

These observations naturally suggest that if the spleen has such an important influence on fat and lipoid metabolism, this mechanism may in some way be concerned in the changes which the spleen effects in the red cells. That the change in the red cells which is responsible for their increased resistance to hemolytic agents after splenectomy is a characteristic of the cells themselves and not a change in the serum, is indicated by the work of Karsner and Pearce,[4] tho it must be admitted in view of King's results that it may be an antihemolytic power of the serum, dependent upon an increase in cholesterin content.

As stated in the first paper[5] of this series, several investigators have noted the occurrence in presumably normal rabbits of positive complement fixation with lipoidal antigens. The reason for this is not known, but we have evidence at hand that lipoidal substances

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[1] Ueber experimentell erzeugte Ablagerungen von Anisotropen Lipoidsubstanz in der Milx und im Knockenmark, Beitr. z. path. Anat. u. allg. Path., 1913, 88, p. 201.

[2] Zur Pathologie der Milzfunktion, Berl. klin. Wchnschr., 1913, 50, pp. 1509, 1572.

[3] Studies in the Pathology of the Spleen, Arch. Int. Med., 1914, 14, p. 145.

[4] The Relation of the Spleen to Blood Destruction and Regeneration and to Hemolytic Jaundice. IV. A Study by the Methods of Immunology of the Increased Resistance of the Red Cells after Splenectomy, Jour. Exper. Med., 1912, 16, p. 769.

[5] Jour. Infect. Dis., 1916, 18, p. 20.

in the serum are concerned in this process, and in view of the well-known importance of lipoids in the Wassermann reaction, it occurred to us, in connection with Anitschkow's observations, as well as those of Eppinger and King, that if in the sera of rabbits showing this phenomenon the reaction became negative after splenectomy, it might be possible by chemical analysis of erythrocytes and serum, and by influencing the reaction through feeding experiments, to throw some light upon the relation of the spleen to normal hemolysis and also upon the question of the factors responsible for increased resistance of the erythrocytes after splenectomy. Our results have not supported our hypothesis; for, altho splenectomy does cause weakening and sometimes a temporary disappearance of the property in normal dog and rabbit serum of non-specific complement fixation, the important factor in this appears to be the anesthetic and not the absence of the spleen. Our observations, however, are of some interest in connection with the problems concerning the mechanism of the Wassermann reaction, and are for that reason presented in brief at this time.

In the present communication are presented only observations of the influence of splenectomy and various anesthetics on the process of non-specific complement fixation in normal rabbit and dog sera. Other antilytic studies and feeding experiments will be published in the fourth paper of this series.

TECHNIC AND METHOD OF STUDY

The rabbits were kept on a constant diet of cabbage, oats, and hay; the dogs were given the usual mixed diet of meats, bread, and vegetables.

By means of preliminary complement-fixation tests, animals the sera of which yielded strongly positive reactions with the antigens used were selected for these studies. Following these selections, the usual procedure was to secure a specimen of blood immediately before anesthesia and operation, and again at varying intervals after operation. The sera were tested in the same dosage, with the same antigens, and with the same technic as in the pre-operative tests; in this manner the influence of the anesthetic and splenectomy upon the serum reactions was studied.

The complement-fixation tests were conducted with sera in a fresh, active state, and again after heating or inactivation at 56 C. for half an hour. The sera were generally used in dosage of 0.1 c.c.; a few experiments were conducted with 0.2 c.c. The dosage of serum appears to us to be important, especially after inactivation, as with the larger dose the percentage of positive reactions is higher and the influence of various factors less marked.

All sera were examined with the 3 lipoidal extracts used in our regular routine Wassermann reactions: an alcoholic extract of beef heart re-enforced with cholesterin; an alcoholic extract of syphilitic liver; and an extract

of acetone insoluble lipoids of human heart. All these extracts were used in dosage equal to twice their antigenic units as determined by titration, these doses being from 6 to 12 times less than their anticomplementary units. In this manner false positive reactions due to antilytic properties of the antigens were avoided. In addition to these lipoidal extracts, 2 bacterial antigens of staphylococci and Bacillus coli communis from human sources were used in our later experiments because of the previous observations that a large percentage of normal rabbit and dog sera absorbed complement with various indifferent and non-specific bacterial antigens. These antigens were titrated prior to each set of reactions and used in the main tests in doses representing one quarter of their anticomplementary units.

The antisheep hemolytic system was employed. Complement was furnished by the pooled sera of at least 2 guinea-pigs and used in dosage of 1 c.c. of a 1:20 dilution (= 0.05 c.c. undiluted serum). The hemolysin was titrated in ascending doses against this constant dose of complement and corpuscle suspension (1 c.c. of a 2.5%) and 2 hemolytic units in the tests proper.

TABLE 1

INFLUENCE OF ETHER ANESTHESIA AND SPLENECTOMY ON NON-SPECIFIC COMPLEMENT FIXATION WITH NORMAL RABBIT SERUM

Rabbit	Reaction Before Operation								
		Active Serum				Inactivated Serum			
	Date	Cholesterinized Alcoholic Extract of Beef Heart	Alcoholic Extract of Syphilitic Liver	Acetone Insoluble Lipoids of Human Heart	Serum Control	Cholesterinized Alcoholic Extract of Beef Heart	Alcoholic Extract of Syphilitic Liver	Acetone Insoluble Lipoids of Human Heart	Serum Control
1	Feb. 6	—	—	—	—	++++	++	++	—
3	Feb. 6	—	—	—	—	+	++	±	—
6	Feb. 19	—	—	—	—	++++	++++	++++	—
	Mar. 4	—	—	—	—	++++	++++	++++	—
7	Feb. 19	—	—	—	—	++++	++++	++++	—
	Mar. 4	—	—	—	—	++++	++++	++++	—
9	Feb. 19	—	—	—	—	++++	++++	++++	—
15	Feb. 6	—	—	—	—	++++	—	—	—
42†	April 6	—	—	—	—	++++	++++	++++	—
	April 8	—	—	—	—	+++	++	++	.....

† Serum from Rabbit 42 was tested in dosage of 0.2 c.c. with all antigens.

KEY TO TABLES

++++ = complete inhibition of hemolysis (strongly positive).
+++ = 75% inhibition of hemolysis (moderately positive).
++ = 50% inhibition of hemolysis (weakly positive).
+ = 25% inhibition of hemolysis (very weakly positive).
± = less than 25% inhibition of hemolysis.
D. H. = delayed hemolysis.
— = complete hemolysis.

As usual antigen, hemolytic, and corpuscle controls were included and invariably a serum control on each serum. The latter is especially important in conducting complement-fixation tests with dog sera because of their tendency to present antihemolytic properties.

All tests were made bi-weekly at the same time as our regular Wassermann reactions, which carefully controlled the antigens and hemolytic system.

INFLUENCE OF ETHER ANESTHESIA AND SPLENECTOMY ON NON-SPECIFIC COMPLEMENT FIXATION WITH NORMAL RABBIT SERUM

The results are shown in Table 1. As a general rule, the operation of splenectomy required from 12 to 20 minutes, during which the animal was under full ether anesthesia.

TABLE 1—*Continued*

INFLUENCE OF ETHER ANESTHESIA AND SPLENECTOMY ON NON-SPECIFIC COMPLEMENT FIXATION WITH NORMAL RABBIT SERUM

Date of Operation	Date	Reaction After Operations							
		Active Serum				Inactivated Serum			
		Cholesterinized Alcoholic Extract of Beef Heart	Alcoholic Extract of Syphilitic Liver	Acetone Insoluble Lipoids of Human Heart	Serum Control	Cholesterinized Alcoholic Extract of Beef Heart	Alcoholic Extract of Syphilitic Liver	Acetone Insoluble Lipoids of Human Heart	Serum Control
Feb. 16	Feb. 23	—	—	—	—	—	—	—	—
	Mar. 4	—	—	—	—	++++	+++	++	—
	Mar. 9	—	—	—	—	++++	+++	+++	—
Feb. 16	Feb. 23	—	—	—	—	—	—	—	—
	Mar. 4	—	—	—	—	+	+++	+	—
	Mar. 9	—	—	—	—	+	+++	+	—
Mar. 7	Mar. 9	—	—	—	—	++++	++++	++	—
	Mar. 16	—	—	—	—	++++	+++	+++	+++
	Mar. 23	+	+	+	—	—	+	+	—
Mar. 7	Mar. 9	—	—	—	—	—	—	—	—
	Mar. 16	—	—	—	—	—	—	—	—
	Mar. 23	—	—	—	—	—	—	—	—
Feb. 26	Mar. 4	—	—	—	—	++++	++++	++++	—
	Mar. 9	—	—	—	—	++++	+++	±	—
	Mar. 16	—	—	—	—	++	++	+	—
	Mar. 23	—	—	—	—	++	+++	+	—
Feb. 16	Feb. 23	—	—	—	—	—	—	—	—
	Mar. 4	—	—	—	—	+++	—	—	—
—	April 8	—	—	—	—	++	++	++	—
	April 10	—	—	—	—	++	++	++	—
	April 13	—	—	—	—	++	++	++	—

As shown in the table, 7 rabbits were examined before, and at intervals after, operation. Of these the sera of 4 (Rabbits 1, 3, 7, and 15) did not fix complement during the following week or 10 days, while 2 (6 and 42) showed a somewhat weaker reaction, and one (9) no effect at all.

INFLUENCE OF ETHER ANESTHESIA AND SPLENECTOMY AND ETHER ANESTHESIA ALONE ON NON-SPECIFIC COMPLEMENT FIXATION WITH NORMAL DOG SERUM

Similar results were observed in dogs, as shown in Table 2. Three dogs were tested before splenectomy and ether anesthesia; in the sera of all three there was complement fixation to some extent with one or more antigens. After splenectomy serum tests were conducted at

TABLE 2

INFLUENCE OF ETHER ANESTHESIA AND SPLENECTOMY ON NON-SPECIFIC COMPLEMENT FIXATION WITH DOG SERUM

Dogs	Reaction Before Operation								
		Active Serum				Inactivated Serum			
	Date	Cholesterinized Alcoholic Extract of Beef Heart	Alcoholic Extract of Syphilitic Liver	Acetone Insoluble Lipoids of Human Heart	Serum Control	Cholesterinized Alcoholic Extract of Beef Heart	Alcoholic Extract of Syphilitic Liver	Acetone Insoluble Lipoids of Human Heart	Serum Control
14-17	Feb. 10	+	±	—	—	++	+	±	±
14-18	Feb. 10	++	+	—	—	++++	++++	++++	+++
14-19	Feb. 10	+++	+++	+++	—	++++	++++	++++	+++
14- 3	.....	.....	.....	.....	.....	.....	.....	.....	.....
13-88	.....	.....	.....	.....	.....	.....	.....	.....	.....
13-84	.....	.....	.....	.....	.....	.....	.....	.....	.....
13-83	.....	.....	.....	.....	.....	.....	.....	.....	.....
13-87	.....	.....	.....	.....	.....	.....	.....	.....	.....
13-85	.....	.....	.....	.....	.....	.....	.....	.....	.....
13- 9	.....	.....	.....	.....	.....	.....	.....	.....	.....
12-51	.....	.....	.....	.....	.....	.....	.....	.....	.....

varying intervals up to about a month after operation; in all tests complement fixation remained persistently absent or was much weaker. In this respect a more profound influence was noted in dogs after splenectomy under ether anesthesia than in rabbits, and, while the results may not be attributable entirely to the anesthetic, we are inclined to ascribe a large part of the serum changes to this agency, as will be pointed out later in this paper.

In addition, 8 dogs were examined one or more times after splenectomy under ether anesthesia, performed from 1 to 10 months previously. As these animals were splenectomized before this work was begun, serum tests were not made preliminary to the operations, but the sera of these animals yielded in general fewer positive reactions than those observed among a large series of normal dogs (6), altho under the circumstances the changes are not sufficiently pronounced

TABLE 2—*Continued*

INFLUENCE OF ETHER ANESTHESIA AND SPLENECTOMY ON NON-SPECIFIC COMPLEMENT FIXATION WITH DOG SERUM

Date of Operation	Date	Reaction After Operations							
		Active Serum				Inactivated Serum			
		Cholesterinized Alcoholic Extract of Beef Heart	Alcoholic Extract of Syphilitic Liver	Acetone Insoluble Lipoids of Human Heart	Serum Control	Cholesterinized Alcoholic Extract of Beef Heart	Alcoholic Extract of Syphilitic Liver	Acetone Insoluble Lipoids of Human Heart	Serum Control
Feb. 19	Feb. 23	—	—	—	—	—	—	—	—
	Mar. 12	—	—	—	—	+	—	—	—
	Mar. 19	—	—	—	—	—	—	—	—
	Mar. 23	—	—	—	—	—	—	—	—
Feb. 19	Feb. 23	—	—	—	—	±	—	—	—
Feb. 19	Feb. 23	—	—	—	—	—	—	—	—
	Mar. 12	—	—	—	—	—	—	—	—
	Mar. 19	—	D. H.	—	—	—	—	—	—
	Mar. 23	D. H.	—	—	—	D. H.	—	—	—
Feb. 19	Mar. 19	—	—	—	—	—	—	—	—
	April 2	++++	++++	++++	±	++++	++++	++++	+
Feb. 12	Mar. 16	—	—	—	—	—	—	—	—
Jan. 7	Mar. 10	—	—	—	—	—	—	—	—
	April 2	—	—	—	—	—	—	—	—
Dec. 10	Mar. 19	—	—	—	—	—	—	—	—
	April 2	++++	++++	++++	—	++++	++++	++++	±
Dec. 12	Feb. 10	—	—	—	—	+	+	+	±
	Mar. 18	—	—	—	—	—	—	—	—
Dec. 10	Feb. 10	++++	+	—	—	++++	++++	++++	+++
	Mar. 19	—	—	—	—	—	—	—	—
	April 2	±	—	—	—	+	±	—	—
April 9	Feb. 10, 1914	—	—	—	—	—	—	—	—
	Mar. 19	—	—	—	—	—	—	—	—
May 31, '14	Feb. 10, 1914	—	—	—	—	+	+	+	+
	Mar. 19	—	—	—	—	—	—	—	—
	Mar. 26	—	—	—	—	D. H.	—	—	—

to lay particular stress on the possible influence of the removal of the spleen.

As controls on the relation of the absence of the spleen to these results, we have studied the influence of (1) ether anesthesia alone and of ether anesthesia and nephrectomy on the serum reactions of a number of rabbits; (2) splenectomy under chloroform anesthesia and chloroform alone; (3) splenectomy under urethan anesthesia and

TABLE 3

INFLUENCE OF ETHER ANESTHESIA ALONE ON NON-SPECIFIC COMPLEMENT FIXATION WITH NORMAL RABBIT SERUM

Rabbit	Date	Reaction Before Ether Anesthesia											
		Active Serum						Inactivated Serum					
		Cholesterinized Alcoholic Extract of Beef Heart	Alcoholic Extract of Syphilitic Liver	Acetone Insoluble Lipoids of Human Heart	Staphylococci	Colon Bacilli	Serum Control	Cholesterinized Alcoholic Extract of Beef Heart	Alcoholic Extract of Syphilitic Liver	Acetone Insoluble Lipoids of Human Heart	Staphylococci	Colon Bacilli	Serum Control
25	Dec. 10	++	++	—	0	0	—	++++	++++	++++	0	0	—
26	Dec. 10	+++	+++	++	0	0	—	++++	++++	++++	0	0	—
29	Dec. 10	++	++	—	0	0	—	+++	+++	++	0	0	.....
30	Dec. 12	—	—	—	0	0	—	—	—	—	0	0	—
35	Dec. 12	—	--	--	0	0	—	++++	++++	+	0	0	—
38	Dec. 12	—	—	—	0	0	—	—	—	—	0	0	—
1*	Mar. 9	—	—	—	0	0	—	++++	++++	+++	0	0	—
15*	Mar. 4	—	—	—	0	0	—	+++	—	—	0	0	—
18	Mar. 26	+++	+++	+++	0	0	—	+++	+++	+++	0	0	—
34†	Mar. 30	++++	+++	0	++++	++++	—	++++	++++	0	++++	++++	—
	April 1	++++	+++	0	++++	++++	—	++++	++++	0	++++	++++	—
36	Mar. 30	—	—	0	++++	++++	—	++++	+++	0	++++	++++	—
	April 1	—	—	0	++	+++	—	++++	+++	0	++++	++++	—
44	April 8	+++	+++	0	++++	++++	—	++++	++++	0	++++	++++	—
51	April 23	—	—	0	—	—	—	+++	+++	0	+++	+++	—
	May 3	--	—	0	—	—	—	+++	+++	0	+++	+++	—
52	April 23	—	—	0	—	—	—	++++	++++	0	++++	++++	—
	May 3	—	—	0	—	—	—	++++	++++	0	++++	++++	—
54	April 23	—	—	0	—	—	—	++++	++++	0	++++	++++	—
	May 3	—	—	0	—	—	—	++++	++++	0	++++	++++	—

* Rabbits 1 and 15 had been splenectomized (see Table 1).

† The sera of Rabbits 34, 36, 44, 51, 52, and 54 were used in dosage of 0.2 c.c. in all tests.

TABLE 3—*Continued*

INFLUENCE OF ETHER ANESTHESIA ALONE ON NON-SPECIFIC COMPLEMENT FIXATION WITH NORMAL RABBIT SERUM

Duration of Anesthesia	Date	Reaction After Ether Anesthesia											
		Active Serum						Inactivated Serum					
		Cholesterinized Alcoholic Extract of Beef Heart	Alcoholic Extract of Syphilitic Liver	Acetone Insoluble Lipoids of Human Heart	Staphylococci	Colon Bacilli	Serum Control	Cholesterinized Alcoholic Extract of Beef Heart	Alcoholic Extract of Syphilitic Liver	Acetone Insoluble Lipoids of Human Heart	Staphylococci	Colon Bacilli	Serum Control
½ hr.	Dec. 10	++	++	—	0	0	—	+++	+++	++	0	0	—
	Dec. 14	—	—	—	0	0	—	+	+	—	0	0	—
	Dec. 17	+	±	—	0	0	—	++	++	++	0	0	—
½ hr.	Dec. 10	—	—	—	0	0	—	++	++	+	0	0	—
½ hr.	Dec. 10	++	++	—	0	0	—	+++	++	+	0	0	—
	Dec. 14	—	—	—	0	0	—	+	+	—	0	0	—
	Dec. 17	++	—	—	0	0	—	+++	+++	++	0	0	—
½ hr.	Dec. 12	—	—	—	0	0	—	—	—	—	0	0	—
	Dec. 14	—	—	—	0	0	—	—	—	—	0	0	—
	Dec. 17	—	—	—	0	0	—	—	—	—	0	0	—
½ hr.	Dec. 12	—	—	—	0	0	—	++++	++++	+	0	0	—
	Dec. 14	—	—	—	0	0	—	+++	+++	—	0	0	—
	Dec. 17	—	—	—	0	0	—	++++	++++	++++	0	0	—
½ hr.	Dec. 12	—	—	—	0	0	—	—	—	—	0	0	—
	Dec. 14	—	—	—	0	0	—	—	—	—	0	0	—
Mar. 17 1½ hr.	Mar. 23	—	—	—	0	0	—	—	±	—	0	0	—
Mar. 17 ½ hr.	Mar. 23	—	—	—	0	0	—	+	—	—	0	0	—
	April 23	—	—	—	0	0	—	—	—	—	0	0	—
Mar. 30 ½ hr.	April 2	+++	+++	+++	0	0	—	+++	+++	++	0	0	+
	April 6	++	++	++	0	0	—	+++	+++	+++	0	0	—
½ hr.	April 1	++++	++++	0	++++	++++	—	++++	++++	0	++++	++++	—
	April 2	—	—	0	—	+	—	—	—	0	++	+++	—
	April 3	++++	++++	0	++++	++++	—	++++	++++	0	++++	++++	—
	April 6	++++	++++	0	++++	++++	—	++++	++++	0	++++	++++	—
10 min.‡	April 1	—	—	0	—	—	—	++++	++++	0	++++	++++	—
25 min.	April 13	—	—	0	—	—	—	++++	++++	0	+++	+++	—
	April 16	—	—	0	—	—	—	++++	+++	0	+++	+++	—
May 3 § 10 min.	May 3	—	—	0	—	—	—	—	—	0	—	—	—
May 3 10 min.	May 3	—	—	0	—	—	—	++++	++++	0	++++	++++	—
	May 4	—	—	0	—	—	—	++++	++++	0	++++	++++	—
	May 5	—	—	0	—	—	—	++++	++++	0	++++	++++	—
	May 6	—	—	0	—	—	—	++++	++++	0	++++	++++	—
May 3 10 min.	May 3	—	—	0	—	—	—	++++	++++	0	++++	++++	—
	May 4	—	—	0	—	—	—	++++	++++	0	++++	++++	—
	May 5	—	—	0	—	—	—	++++	++++	0	++++	++++	—
	May 6	—	—	0	—	—	—	++++	++++	0	++++	++++	—

‡ Died during a 10-minute period of anesthesia. Blood was at once secured.
§ Died 4 minutes after anesthesia. Blood was at once secured.

urethan alone; (4) splenectomy under nitrous oxid anesthesia and nitrous oxid alone.

INFLUENCE OF ETHER ANESTHESIA ALONE ON NON-SPECIFIC COMPLEMENT FIXATION WITH NORMAL RABBIT SERUM

Fifteen rabbits in all were examined before, and at intervals after, ether anesthesia varying from 10 to 30 minutes in duration. The results are shown in Table 3.

1. The sera of 5 rabits (25, 29, 1, 15, and 34) showed a definite weakening or absence of complement fixation, with both active and inactivated sera, at some time during a brief period following ether anesthesia.

2. The sera of 3 rabbits (26, 36, and 44) showed changes when tested in an active state but not after heating or inactivation.

TABLE 4

INFLUENCE OF ETHER ANESTHESIA AND SPLENECTOMY ON NON-SPECIFIC COMPLEMENT FIXATION WITH NORMAL RABBIT SERUM

Rabbit	Reaction Before Operation								
		Active Serum				Inactivated Serum			
	Date	Cholesterinized Alcoholic Extract of Beef Heart	Alcoholic Extract of Syphilitic Liver	Acetone Insoluble Lipoids of Human Heart	Serum Control	Cholesterinized Alcoholic Extract of Beef Heart	Alcoholic Extract of Syphilitic Liver	Acetone Insoluble Lipoids of Human Heart	Serum Control
18	Mar. 26	+++	+++	+++	—	+++	+++	+++	—
21	Mar. 30	++++	++++	++++	—	++++	++++	++++	+++
	April 2	++++	++++	++++	—	++++	++++	++++	++

3. The sera of 4 rabbits (35, 18, 52 and 54) did not appear to be affected at all.

4. The influence of ether alone is only slight immediately after the anesthesia, and is usually most marked after several days. Gradually the serum returns to its former condition in relation to non-specific complement fixation.

5. As already pointed out, changes in the power of rabbit serum to give these non-specific complement fixations due to ether anesthesia are more apparent when it is used in a fresh, active state; likewise, in a number of reactions it would appear that of the lipoidal extracts the reaction with an alcoholic extract of heart re-enforced with cholesterin showed least change, and the altered power of

reaction of a serum was less in evidence with the bacterial antigens than with the lipoidal antigens.

It may be noted in this connection that the effect of ether anesthesia upon rabbit serum in the Wassermann reaction is directly opposite to that described as occurring with human serum, inasmuch as a positively reacting rabbit serum tends to become negative. The mechanism of non-specific complement fixation with rabbit and dog sera, however, presents several features differing widely from that concerned in the Wassermann reactions with human serum, so that, while no doubt somewhat related, they are not identical.

It would appear, therefore, that the results obtained after splenectomy are due to anesthesia rather than to removal of the spleen. This supposition was strengthened when nephrectomy under ether anesthesia in 2 rabbits showed similar changes (Table 4).

TABLE 4—*Continued*

INFLUENCE OF ETHER ANESTHESIA AND NEPHRECTOMY UPON NON-SPECIFIC COMPLEMENT FIXATION WITH NORMAL RABBIT SERUM

Date of Operation	Reaction After Operation								
		Active Serum				Inactivated Serum			
	Date	Cholesterinized Alcoholic Extract of Beef Heart	Alcoholic Extract of Syphilitic Liver	Acetone Insoluble Lipoids of Human Heart	Serum Control	Cholesterinized Alcoholic Extract of Beef Heart	Alcoholic Extract of Syphilitic Liver	Acetone Insoluble Lipoids of Human Heart	Serum Control
April 11	April 23	—	—	—	—	—	—	—	—
	May 5	—	—	—	—	—	—	—	—
	May 11	—	—	—	—	—	—	—	—
	Oct. 16	—	—	—	—	—	—	—	—
April 7	April 13	—	—	—	—	+++	++	+	—
	April 23	+++	++	++	—	+++	++	++	—

INFLUENCE OF CHLOROFORM ANESTHESIA AND SPLENECTOMY AND CHLOROFORM ANESTHESIA ALONE ON NON-SPECIFIC COMPLEMENT FIXATION WITH NORMAL RABBIT SERUM

Changes similar to those following ether anesthesia were apparent after the administration of chloroform. Our series, however, does not include a large number of animals because of the difficulty in giving chloroform to rabbits over a prolonged period of time.

The results in serum changes following one splenectomy under chloroform anesthesia and those following chloroform anesthesia alone are shown in Table 5. Unfortunately the splenectomized animal succumbed soon after operation, so that a series of examinations could not

be made. While in this rabbit changes in the reacting power of the serum occurred—positive reactions with the lipoidal antigens becoming negative, and those with the bacterial antigens weaker after the anesthesia and operation—the influence of chloroform alone was somewhat less apparent than had been the influence of ether alone, probably, to some extent, on account of the shorter period of anesthesia.

TABLE 5

INFLUENCE OF CHLOROFORM ANESTHESIA AND SPLENECTOMY AND CHLOROFORM ANESTHESIA ALONE ON NON-SPECIFIC COMPLEMENT FIXATION WITH NORMAL RABBIT SERUM

Rabbit	Date	Reaction Before Anesthesia and Operation											
		Active Serum						Inactivated Serum					
		Cholesterinized Alcoholic Extract of Beef Heart	Alcoholic Extract of Syphilitic Liver	Acetone Insoluble Lipoids of Human Heart	Staphylococci	Colon Bacilli	Serum Control	Cholesterinized Alcoholic Extract of Beef Heart	Alcoholic Extract of Syphilitic Liver	Acetone Insoluble Lipoids of Human Heart	Staphylococci	Colon Bacilli	Serum Control
43*	April 6	0	0	0	0	0	0	++++	++++	0	++++	++++	—
	April 8	—	—	0	—	—	—	++++	++++	0	++++	++++	—
39	Mar. 30	—	—	0	++++	++	—	++	++	0	++++	++	.....
	April 1	—	—	0	—	—	—	+++	+++	0	++++	+++	—
40	Mar. 30	—	—	0	++++	++	—	+	++	0	++++	++	—
	April 1	—	—	0	++++	++	—	+	++	0	++++	++++	—
45	April 8	--	—	0	—	—	—	++	++	0	++++	++++	—

* All tests with the sera of these rabbits were made with 0.2 c.c. amounts.

INFLUENCE OF URETHAN ANESTHESIA AND SPLENECTOMY AND URETHAN ALONE ON NON-SPECIFIC COMPLEMENT FIXATION WITH NORMAL RABBIT SERUM

Urethan was administered in dose of 15 grains to each rabbit by means of a stomach tube. The effects upon the serum reactions were similar to those observed following the administration of ether, probably, in part, on account of a similar solvent action of the drug upon lipoids.

The results of the complement-fixation tests are shown in Table 6. In the two splenectomized animals (Rabbits 24 and 30), the

serum reactions were altered soon after operation, becoming weaker or negative and later running to their former states. Likewise, after the administration of urethan alone (Rabbits 32 and 34), the reactions were much weaker and in this respect were similar to the changes produced by ether, in that the alteration of serum reaction was not immediate, but was apparent one or two days after an interval of 7 to 10 days.

TABLE 5—*Continued*

INFLUENCE OF CHLOROFORM ANESTHESIA AND SPLENECTOMY AND CHLOROFORM ANESTHESIA ALONE ON NON-SPECIFIC COMPLEMENT FIXATION WITH NORMAL RABBIT SERUM

Anesthesia and Duration of Operation	Date	Reaction After Anesthesia and Operation											
		Active Serum						Inactivated Serum					
		Cholesterinized Alcoholic Extract of Beef Heart	Alcoholic Extract of Syphilitic Liver	Acetone Insoluble Lipoids of Human Heart	Staphylococci	Colon Bacilli	Serum Control	Cholesterinized Alcoholic Extract of Beef Heart	Alcoholic Extract of Syphilitic Liver	Acetone Insoluble Lipoids of Human Heart	Staphylococci	Colon Bacilli	Serum Control
Splenectomy†	April 8	—	—	0	—	—	—	—	—	0	++	++++	—
Chloroform 6 min.	April 1	—	—	0	—	—	—	—	—	0	—	—	—
Chloroform 10 min.	April 1	—	—	0	+	+	—	+	+++	0	++++	++++	—
	April 2	—	—	0	+	++	—	0	0	0	0	0	0
	April 3	—	—	0	—	—	—	++	++	0	++++	++++	—
	April 6	—	—	0	—	—	—	+	+	0	+	+	—
	April 10	—	—	0	—	—	—	++	++	0	++++	++++	—
	April 13	—	—	0	—	—	—	++	++	0	++++	++++	—
	April 16	—	—	0	—	—	—	++	++	0	++	++	—
Chloroform 10 min.	April 8	—	—	0	—	—	—	±	±	0	++++	++++	—
	April 13	—	—	0	—	—	—	++	++	0	++++	++++	—
	April 16	—	—	0	—	—	—	+++	++	0	++++	++++	—

† Duration of anesthesia 15 minutes.

INFLUENCE OF NITROUS OXID ANESTHESIA AND SPLENECTOMY AND NITROUS OXID ANESTHESIA ALONE ON NON-SPECIFIC COMPLEMENT FIXATION WITH NORMAL RABBIT SERUM

In order to settle more definitely the question of the influence of removal of the spleen on the phenomenon of non-specific complement fixation in normal rabbit serum, 2 positively reacting animals were splenectomized under nitrous oxid oxygen anesthesia; in this manner we avoided using anesthetics that are lipoid solvents, as ether, chloroform, and urethan. These operations required about 12 minutes each,

during which the animals were completely anesthetized. For controls, 2 other positively reacting rabbits were anesthetized in the same manner and for the same length of time.

Serum tests were made immediately before and after the anesthesia and operations, and again at intervals of several days. No appreciable changes in the reaction followed splenectomy under nitrous oxid oxygen anesthesia. Likewise, the anesthetic alone had no influence upon the

TABLE 6

INFLUENCE OF URETHAN ANESTHESIA AND SPLENECTOMY AND URETHAN ANESTHESIA ALONE ON NONSPECIFIC COMPLEMENT FIXATION WITH NORMAL RABBIT SERUM

Rabbit	Reaction Before Anesthesia and Operation								
	Date	Active Serum				Inactivated Serum			
		Cholesterinized Alcoholic Extract of Beef Heart	Alcoholic Extract of Syphilitic Liver	Acetone Insoluble Lipoids of Human Heart	Serum Control	Cholesterinized Alcoholic Extract of Beef Heart	Alcoholic Extract of Syphilitic Liver	Acetone Insoluble Lipoids of Human Heart	Serum Control
24	Nov. 3	—	—	—	—	++++	++	++	—
30	Nov. 3	—	—	—	—	++++	++++	++++	—
32	Nov. 3	—	—	—	—	+++	+++	++	—
34	Nov. 3	—	—	—	—	++++	++	++	—

serum reactions. The conclusion seems warranted, therefore, that the results observed after splenectomy under ether, chloroform, and urethan are to be ascribed to the anesthetic used rather than to the removal of the spleen itself and are due probably to the effects of the former upon serum lipoids.

CONCLUSIONS

Anesthetics as ether, chloroform, and to a slight extent urethan, generally weaken or remove temporarily the power in normal rabbit

and dog sera of absorbing or fixing complement with lipoidal and bacterial antigens in a non-specific manner. This alteration usually is not apparent at once after the administration of the anesthetic, but is found after 1 to 3 days; later the serum returns to its former power of causing this non-specific complement fixation.

The administration of ether does not alter negatively reacting sera in such manner as to bring about positive reactions.

TABLE 6—*Continued*

INFLUENCE OF URETHAN ANESTHESIA AND SPLENECTOMY AND URETHAN ANESTHESIA ALONE ON NON-SPECIFIC COMPLEMENT FIXATION WITH NORMAL RABBIT SERUM

Date of Operation	Reaction After Anesthesia and Operation								
		Active Serum				Inactivated Serum			
	Date	Cholesterinized Alcoholic Extract of Beef Heart	Alcoholic Extract of Syphilitic Liver	Acetone Insoluble Lipoids of Human Heart	Serum Control	Cholesterinized Alcoholic Extract of Beef Heart	Alcoholic Extract of Syphilitic Liver	Acetone Insoluble Lipoids of Human Heart	Serum Control
Splenectomy Nov. 7	Nov. 7	—	—	—	—	—	+	—	—
	Nov. 8	—	—	—	—	—	+	—	—
Splenectomy Nov. 7	Nov. 7	—	—	—	—	++	+++	++	—
	Nov. 9	—	—	—	—	—	—	—	—
	Nov. 13	—	—	—	—	—	++	—	—
	Nov. 16	0	0	0	0	+	+++	+++	—
	Nov. 23	0	0	0	0	+++	++	++	—
Anesthesia only Nov. 7	Nov. 7	—	—	—	—	+++	+++	+++	—
	Nov. 8	—	—	—	—	+	+	—	—
Anesthesia only Nov. 7	Nov. 7	—	—	—	—	++	++	++	—
	Nov. 9	—	—	—	—	—	+	—	—
	Nov. 13	—	—	—	—	+	+	+	—
	Nov. 16	0	0	0	0	++	++	±	—
	Nov. 19	0	0	0	0	++++	++++	+++	—

Nitrous oxid oxygen anesthesia has no appreciable influence on the serum reactions of normal rabbits.

Splenectomy alone has probably no influence upon the property in normal rabbit and dog sera of fixing or absorbing complement with various non-specific lipoidal and bacterial antigens, the effects being in larger degree attributable to the anesthetic; the changes observed in dogs following splenectomy under ether were somewhat more profound than those in rabbits.